RESPONSE

Claims 1-10, 13-16, 26, and 28-32 are pending in the application. Claims 1-10, 13-16, 26, and 28-32 were rejected under 35 U.S.C. §103 as described below. Applicant respectfully requests reconsideration of the application and claims in light of the following remarks.

Rejection under 35 U.S.C. § 103(a) over Harrington *et al.* and Prusiner *et al.*

Claims 1 and 2 were rejected under 35 U.S.C. 103(a) as being obvious over U.S. Patent No. 4,892,814 to Harrington *et al.* ("Harrington *et al.*") in view of PCT Intl. Pub. No. WO 95/31466 by Prusiner *et al.* ("Prusiner *et al.*"). In particular, the Office Action suggests that:

> Harrington *et al.* teach a method for typing a sample of a prion or spongiform encephalopathy disease or Creutzfeldt-Jakob disease, the method comprising comparing and identifying similar physicochemical properties of the sample with a standard of known type . . .
>
> Harrington *et al.* teach a method for assessing and predicting the susceptibility of a human to bovine spongiform encephalopathy or a derivative thereof . . .
>
> Prusiner *et al.* teach the sizes and ratios of distinct PrP Sc type glycoforms . . .
>
> It would have been *prima facie* obvious . . . to substitute and combine the sizes and ratios of distinct PrP Sc type glycoforms of Prusiner *et al.* in the method for typing a sample of a prion or spongiform encephalopathy of Harrington *et al.* since Prusiner *et al.* state, "It appears that the scrapie isoform of the prion protein (PrPSc) is necessary for both transmission and pathogenesis of the transmissible neurodegenerative diseases of animals and humans . . . "

Respectfully, Applicant must disagree with the characterization of these references in the Office Action, and the conclusion that it would have been obvious to combine their teachings to produce the present invention.

Harrington et al. Does Not Compare or Identify PrP Proteins

The experiments disclosed in the Harrington *et al.* reference do not, in fact, compare or identify PrP proteins. Rather, Harrington *et al.* assayed the proteins present in spinal fluid

samples obtained from patients with a variety of neurological and medical conditions, including Creutzfeldt-Jakob disease (CJD). They identified two proteins, which they designated 130 and 131, which were present in all CJD patients, and two other proteins, designated 127 and 128, which were present in 67% of CJD patients. However, Harrington *et al.* teach at column 5, lines 36-43 (emphasis added):

> The relative mass of the cerebrospinal fluid proteins 130 and 131 is similar to that of the PrP 27-30 complex, but the complex has a much more basic pI. An antiserum to PrP 27-30 was used to probe Western blots containing proteins 127, 128, 130, and 131. Since no reactivity was detected, proteins 127, 128, 130, and 131 appear to be different from the scrapie PrP protein.

Moreover, Hsich *et al.* (1996), *N. Engl. J. Med.* 335(13): 924-930 ("Hsich *et al.*") reports the identification of proteins 130 and 131 as corresponding to a single protein designated as 14-3-3. Although the protein has diagnostic potential, it is mainly a marker of brain injury and a marker of brain disease in general. The 14-3-3 protein is not the human PrP protein and, therefore, is not specific for prion or spongiform encephalopathy disease or Creutzfeldt-Jakob disease. (A copy of the Hsich *et al.* reference is submitted herewith.)

Therefore, Harrington *et al.* do not teach or suggest "comparing and identifying similar physicochemical properties of the sample with a standard sample of known PrP^{Sc} type" as claimed in claims 1 and 2 (emphasis added). Rather, they teach determining the presence or absence of a non-PrP protein.

Harrington et al. Does Not Teach or Suggest Comparisons to PrP^{Sc} Standards

Furthermore, Harrington *et al.* do not teach or suggest a method of typing a prion or spongiform encephalopathy disease or Creutzfeldt-Jakob disease by "comparing and identifying similar physicochemical properties of the sample with a standard sample of known [disease-associated protein] type" as required by claims 1 and 2. That is, not only do Harrington *et al.* fail to compare and identify PrP or PrP^{Sc} proteins, they also fail to compare a sample protein with any standard sample of a disease-associated protein at all.

Rather, Harrington *et al.* subjected the proteins present in spinal fluid samples to electrophoresis and used molecular mass standards obtained from a commercial source to obtain the molecular mass (Mr) of the proteins in the sample (column 3, lines 63-68). Next, they subjected the proteins to isoelectric focusing electrophoresis to determine the isoelectric point (pI) of the proteins in the sample (column 3, line 68 - column 4, line 4). They found that the 130 and 131 proteins were present in all CJD patients and 50% of herpes simplex encephalitis patients, but were absent in patients with a variety of other neurological and medical conditions (Table 1). In addition, the 127 and 128 proteins were present in 67% of CJD patients and 50% of herpes simplex encephalitis patients, but were absent in patients with a variety of other neurological and medical conditions (Table 1).[1]

Significantly, although Harrington *et al.* identified four proteins whose presence or absence was indicative of CJD, in none of the experiments of Harrington *et al.* were the "physicochemical properties of the sample [compared] with a standard sample of known [disease-associated] type" as required by claims 1 and 2. Instead, the molecular masses of the proteins present in the sample were compared to commercially available molecular mass standards, and not to disease-associated protein standards. Moreover, the molecular masses and isoelectric points were used only to determine the presence or absence of the proteins, and not to identify the forms or types of the proteins present.

Therefore, Applicant submits that Harrington *et al.* not only fail to teach or suggest the comparison and identification of CJD-associated PrP or PrP^{Sc} proteins, but they also fail to teach or suggest the comparison and identification of any sample proteins with the corresponding disease-associated types of those proteins.

Prusiner et al. and Harrington et al. Are Not Properly Combined

The Prusiner *et al.* reference is directed to the production of transgenic animals (e.g., mice) expressing chimeric PrP proteins, particular human-murine chimeric proteins designated MHu2MPrP. The chimeric proteins are fusions comprising sequences from a first species (e.g.,

[1] Harrington *et al.* also conducted experiments to determine whether proteins 127, 128, 130 or 131 could be derived from normal spinal fluid proteins by proteolytic degradation or post-translational modification (column 4, lines 5-19). These experiments were negative (column 4, lines 63-68).

human), which is to be tested for a particular prion disease, and a second species (e.g., mouse), which is to be used as the transgenic diagnostic animal model. Thus, the animals of the second species are transgenic for a species-specific form of chimeric PrP (e.g., human-murine, bovine-murine, ovine-murine), and develop prion disease at rates above background levels when infected by a species-specific form of PrP^{Sc} from the first species. The transgenic animals are used as diagnostic tools to determine whether an animal of the first species is infected with a pathogenic prion by injecting a sample from the animal of the first species into the transgenic animal, and determining whether the transgenic animal develops the prion disease.

There is nothing, however, in the Prusiner *et al.* reference that teaches or suggests "comparing and identifying similar physicochemical properties of the sample with a standard sample of known PrP^{Sc} type" as claimed in claims 1 and 2. Rather, the authors teach the use of transgenic animal models to identify the presence of a PrP^{Sc} protein in a sample by injecting the animal with the sample and determining, either clinically or histologically *post mortem*, whether prion disease results. The disclosed methods determine only whether the sample was infected with the relevant PrP^{Sc} protein, and do not determine whether there are different forms or types of PrP^{Sc} present in the sample. This is distinct from the claimed invention, which differentiates between the different forms or types of PrP^{Sc} by comparing the physicochemical properties of the sample to a standard sample of known PrP^{Sc} type.

Therefore, Applicant submits that there is no motivation to combine the teachings of Prusiner *et al.*, which is directed to *in vivo* transgenic animal assays and clinical or histological analysis, with the teachings of Harrington *et al.*, which is directed to *in vitro* assays and electrophoretic weight and isoelectric point analysis. Therefore, Applicant respectfully submits that the combination of Harrington *et al.* and Prusiner *et al.* is improper and requests that the rejection based upon Harrington *et al.* and Prusiner *et al.* be reconsidered and withdrawn.

Prusiner et al. Does Not Disclose the Physicochemical Properties of PrP^{Sc} Types

Finally, the Office Action suggests that "Prusiner *et al.* teach the sizes and ratios of distinct PrP Sc type glycoforms" and cites to page 2, lines 3-6, and Examples 8-9 of the Prusiner

et al. reference. Respectfully, Applicant has carefully reviewed the cited passages and cannot identify this teaching in Prusiner *et al.*

Specifically, page 2, lines 3-6 reads as follows:

> It appears that the scrapie isoform of the prion protein (PrPSc) is necessary for both the transmission and pathogenesis of the transmissible neurodegenerative diseases of animals and humans.

There is nothing, however, in this passage, which teaches one of skill in the art anything about the physicochemical properties of different types of PrP^{Sc} proteins, much less the "sizes and ratios of distinct PrP Sc type glycoforms".

Example 8 of Prusiner *et al.* describes the production of two lines of transgenic mice expressing the human PrP protein, which Prusiner *et al.* designate HuPrP. They disclose that the transgenic mice express HuPrP at 4-8-fold the level found in human brain, but that the mice are no more susceptible to human prions (PrP^{Sc}) than non-transgenic mice. In contrast, transgenic mice expressing a fusion protein of the murine and human prion proteins, MHu2MPrP, were significantly more likely to develop prion disease after injection with human PrP^{Sc}.

To confirm the diagnosis of prion disease, the diseased brains of transgenic HuPrP mice, designated Tg(HuPrP), and non-transgenic (non-Tg) mice were tested with antibodies against the HuPrP protein and the mouse equivalent, MoPrP. The authors note that "the protease-resistant fragments of the $HuPrP^{Sc}$ from ill Tg(HuPrP) mice migrate more rapidly on SDS-PAGE than do those of $HuPrP^{CJD}$ from human CJD brain and $MoPrP^{Sc}$ from non-Tg mouse brain." This is not, however, a comparison of the "physicochemical properties of [a] sample with a standard sample of known PrP^{Sc} type". Rather, a human protein expressed in a diseased transgenic mouse is compared with a human protein expressed in a diseased human and a mouse protein expressed in a diseased mouse. There is no comparison of the same protein expressed in both diseased and normal individuals (or in test and control individuals), and there is no teaching or suggestion of using such a comparison for typing a prion or spongiform encephalopathy disease. Instead, the comparison is part of an evaluation of the transgenic mouse model itself, and not a method of diagnosis.

Moreover, there is nothing in Example 8 which teaches or suggests to one of skill in the art anything about the physicochemical properties of different form or types of a PrP^{Sc} protein that could be used for comparison or identification in a diagnostic method, much less the "sizes and ratios of distinct PrP Sc type glycoforms". If anything, Example 8 of Prusiner *et al.* teaches that a human PrP^{Sc} protein expressed in a diseased Tg(HuPrP) mouse cannot be used as a standard sample for comparison in diagnosis.

Example 9 of Prusiner *et al.* describes the histopathological analysis of the brains of Tg(MHu2M) mice which were injected with human prion disease proteins and which subsequently developed prion disease. The analysis revealed the presence of protease-resistant PrP^{Sc} proteins in the brains. One of the antibodies employed by the authors recognizes an epitope present in the human-murine chimeric protein MHu2M but not in the naturally-occurring MoPrP protein. Others were "poorly reactive" with either chimeric or human prion disease proteins.

There is nothing, however, in Example 9 of Prusiner *et al.* which teaches or suggests to one of skill in the art anything about the physicochemical properties of different types of PrP^{Sc} proteins, much less the "sizes and ratios of distinct PrP Sc type glycoforms". Instead, the detection of the proteins is merely a positive control that the transgenic animal model has worked, and the presence of the PrP^{Sc} in the animals is merely a confirmation of the clinical and histological diagnosis that the animals have been infected.

Therefore, Applicant respectfully submits that the Prusiner *et al.* reference does not, in fact, teach the sizes and ratios of distinct PrP^{Sc} types.

The Claimed Invention is Not Obvious Over Harrington et al. and Prusiner et al.

In light of the foregoing, Applicant respectfully submits that the Harrington *et al.* and Prusiner *et al.* references, even if combined, do not produce the claimed invention and, therefore, Applicant requests that the rejection based upon Harrington *et al.* and Prusiner *et al.* be reconsidered and withdrawn.

Rejection under 35 U.S.C. § 103(a) over Race *et al.* and Prusiner *et al.*

Claims 1, 3-10, 13-16, 26 and 28-32 were rejected under 35 U.S.C. 103(a) as being obvious over Race *et al.* (1992), *Am. J. Vet. Res.* 53(6):883-889 ("Race *et al.*") in view of Prusiner *et al.* In particular, the Office Action suggests that:

> Race *et al.* teach a method for typing a sample of a prion or spongiform encephalopathy disease, the method comprising comparing and identifying similar physicochemical properties of the sample with a standard of known type . . .
>
> Race *et al.* teach a method wherein the comparison of physicochemical properties comprises a comparison of protease resistance . . .
>
> Race et al. teach a method for identifying infection in an animal and tissue of bovine spongiform encephalopathy, the method comprising isolating a prion protein from the animal and/or tissue and identifying that the prion protein can be characterized by having three distinct bands on an electrophoresis gel following proteinase K digestion . . . the bands comprising I) a band of highest molecular weight in the greatest proportion, ii) a band of lowest molecular weight in the lowest proportion, and iii) a band with a molecular weight between I and ii and of a proportion between I and ii . . .
>
> Prusiner *et al.* teach the sizes and ratios of distinct PrP Sc type glycoforms . . .
>
> It would have been *prima facie* obvious . . . to substitute and combine the sizes and ratios of distinct PrP Sc type glycoforms of Prusiner *et al.* in the method for typing a sample of a prion or spongiform encephalopathy of Race *et al.* since Prusiner *et al.* state, "It appears that the scrapie isoform of the prion protein (PrPSc) is necessary for both transmission and pathogenesis of the transmissible neurodegenerative diseases of animals and humans . . . "

Respectfully, Applicant must disagree with the characterization of these references in the Office Action, and the conclusion that it would have been obvious to combine their teachings to produce the present invention.

Race et al. Does Not Teach or Suggest Comparisons to PrP^{Sc} Standards

Race *et al.* teaches a method for determining the presence or absence of PrP^{Sc} protein in tissue samples from sheep by Western blotting. The methods taught by Race *et al.* involve using an antibody that specifically binds to a prion protein to detect the presence or absence of the

prion protein. Race *et al.* does not address the existence of different forms or types of PrP^{Sc} proteins or the comparison of the physicochemical properties of a sample with a standard sample of known PrP^{Sc} type. Although the Western blots of protease-treated samples presented in Race *et al.* display protein fragments of varying molecular masses, Race *et al.* does not compare the physicochemical properties, specifically the sizes and ratios, of different PrP^{Sc} types.

The Office Action suggests that Race *et al.* teaches a diagnostic method in which prion protein samples are subjected to proteinase K degradation and gel electrophoresis, and that the resulting gels show "three distinct bands" in which the band of highest molecular weight is in the greatest proportion, the band of lowest molecular weight is in the lowest proportion, and the band of intermediate molecular weight is in an intermediate proportion. Respectfully, although Race *et al.* does describe subjecting brain samples to proteinase K degradation and electrophoresis prior to immunoblotting, Applicant has carefully reviewed the cited passages and figures cannot identify any teaching or suggestion in Race *et al.* regarding the three distinct bands and their relative proportions.

Specifically, the legend of Figure 1 reads, in pertinent part, as follows (emphasis added):

> Figure 1 - Representative immunoblot showing lack of or presence of characteristic proteinase K-resistant protein (PrP-res) bands in brain homogenates of clinically normal or clinically affected sheep . . . Samples in lanes 4-6 were derived from cerebellum or diencephalon obtained from sheep that were scrapie-positive clinically, but that could not be confirmed as scrapie-positive histologically. Samples in lanes 7 and 8 were from sheep that were scrapie-positive clinically and histopathologically.

Thus, lanes 4-8 show immunoblots from sheep infected with prion disease. Examination of the figure, however, shows that the immunoblots in each lane differ widely, with lane 4 showing a single strong band and much weaker secondary band of lower molecular weight; lane 5 showing roughly equal strong bands at positions similar to those of lane 4; lane 6 showing stronger versions of the bands of lane 5 as well as an additional distinct dark band at a new lower molecular weight position; and lanes 7 and 8 showing extremely strong indistinct bands spanning the three positions of lane 6 as well as a new band of medium to strong intensity at a higher molecular weight position. In all, there are at least four different band positions and no regular ratio of intensity amongst them.

Similarly, the legend of Figure 5 reads, in pertinent part, as follows (emphasis added):

> Figure 5 - Representative immunoblot showing the presence or absence of PrP-res proteins in sheep mesenteric lymph nodes . . . Lanes 8 and 10 contain tissue from sheep that were scrapie-positive clinically and histologically. Lanes 4-7 and 9 and 11 contain tissue from sheep that had clinical changes consistent with scrapie, but did not have all the necessary histopathological changes to be considered scrapie-positive.

Thus, only lanes 8 and 10 show immunoblots from sheep that were unambiguously infected with prion disease. Examination of the figure, however, shows that the immunoblots in lanes 8 and 10 differ widely, with lane 8 showing a single strong band and much weaker secondary band of lower molecular weight, and lane 10 showing stronger bands at each of the positions of lane 8 as well as a strong additional band of higher molecular weight and a weaker band of lower molecular weight. In all, there are at least four different band positions and no regular ratio of intensity amongst them. There is similarly no pattern amongst lanes 4-7 and 9 and 11, which were from sheep with ambiguous diagnoses with prion disease.

Thus, there are no consistent results in Race *et al.* with respect to the number of bands, the size of fragments, or the ratios of fragments of PrP^{Sc} proteins. Indeed, in Race *et al.*, there is no analysis of the size or patterns of the PrP^{Sc} fragments but, rather, a teaching that the mere presence of immunoreactive bands provides a positive diagnosis of scrapie infection. In contrast, the present application teaches that there can be an improved diagnosis by comparing the different sizes and ratios of sizes of the bands to standard samples of known PrP^{Sc} types.

Thus, Applicant respectfully submits that Race *et al.* does not teach a method that determines the type of PrP^{Sc} protein present in a sample by comparing and identifying similar physicochemical properties of a sample with a standard sample of known type, as required by claim 1.

Prusiner et al. Does Not Disclose the Physicochemical Properties of PrP^{Sc} Types

The Office Action suggests that "Prusiner *et al.* teach the sizes and ratios of distinct PrP Sc type glycoforms" and can be combined with Race et al. to produce the claimed invention. As discussed above with respect to the rejections based on Harrington *et al.* and Prusiner *et al.*,

Applicant respectfully submits that the Prusiner *et al.* reference does not, in fact, teach the sizes and ratios of distinct PrP^{Sc} types.

The Claimed Invention is Not Obvious Over Race et al. and Prusiner et al.

In light of the foregoing, Applicant respectfully submits that the Race *et al.* and Prusiner *et al.* references, even if combined, do not produce the claimed invention and, therefore, Applicant requests that the rejection based upon Race *et al.* and Prusiner *et al.* be reconsidered and withdrawn.

CONCLUSION

Applicant respectfully requests reconsideration of the application in light of the foregoing remarks and the Hsich *et al.* reference. If the Examiner believes that a telephonic interview would expedite the allowance of the application, the Examiner is invited to contact the undersigned attorney at the number below.

A Request for Continued Examination and a petition for a three-month extension of time for response is submitted herewith. The Commissioner is authorized to debit the $375.00 fee for the Request for Continued Examination and the $465.00 fee for the extension of time, as well as any other fee necessary to maintain the pendency of this application, from Deposit Account No. 08-0219.

Respectfully submitted,
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